REMARKS/ARGUMENTS

The Objection to the Specification Should Be Withdrawn

The specification has been objected to for containing an embedded hyperlink and/or other form of browser executable code on page 23 at line 15. The Office indicates that Applicant is required to delete the embedded hyperlink and/or other form of browser executable code.

Applicants have amended the specification to delete the embedded hyperlink and/or other form of browser executable code on page 23 at line 15. Accordingly, the objection to the specification should be withdrawn.

Status of the Claims

Claims 62, 63, 67, 68, 72-74, 87, and 88 have been amended. Claims 80-86 have been canceled without prejudice or disclaimer. Applicants expressly reserve the right to file one or more continuing applications directed to the subject matter of the canceled claims.

Claims 62, 67, 73, and 87 have been amended to point out more distinctly that the nucleotide sequences of parts (c) and (d) encode proteins comprising NADPH-thioredoxin reductase activity. Part (d) of these claims has been further amended to recite specific stringent hybridization conditions and to clarify that the nucleotide sequence "hybridizes under stringent conditions to *the complement of* the nucleotide sequence set forth in SEQ ID NO: 24 . . . ." (emphasis added) Support for these amendments to the claims can be found in the specification, particularly page 14 at lines 11-13, page 16 at lines 12-14, page 17 at lines 18-21, page 19 at lines 26-31, page 20, and page 21 at lines 1-22.

Claims 63, 68, 74, and 88 have been amended to point out more distinctly that the nucleotide sequences of parts (c) and (d) encode proteins comprising thioredoxin *h* activity. Part (d) of these claims has been further amended to recite specific stringent hybridization conditions and to clarify that the nucleotide sequence "hybridizes under stringent conditions to *the*

complement of the nucleotide sequence set forth in SEQ ID NO: 13 . . . ." (emphasis added) Support for these amendments to the claims can be found in the specification, particularly page 14 at lines 11-13, page 16 at lines 12-14, page 17 at lines 18-21, page 19 at lines 26-31, page 20, and page 21 at lines 1-22.

Claim 72 has been amended to in response to a claim objection as described in detail below. Support for the amendment to claim 72 can be found in original claim 34 and in the specification, particularly on page 3 at lines 29-30, page 4 at lines 1-2, page 9 at lines 8-14, page 44 at lines 19-22, and page 55 at lines 16-19.

Claim 87 has been further amended to correct an obvious clerical error at claim line 2. In this claim, the indefinite article "a" was repeated in the recitation "genome a a first nucleotide construct". Accordingly, the second occurrence of "a" was deleted from this recitation.

No new matter has been added by way of the amendments to the claims.

Claims 62-79 and 87-94 are pending.

Reexamination and reconsideration of the application as amended are respectfully requested in view of the following remarks.

The Objection to Claim 72 Should Be Withdrawn

Claim 72 has been objected to for reciting at line 2 "plant corn" instead of "corn plant". Applicants have amended claim 72 at line 2 to correct an obvious clerical error by deleting the recitation of "plant" that immediately follows "corn" and then inserting the word --plant-- immediately before "corn". Additionally, Applicants have further amended this claim at line 2 to correct another obvious clerical error by inserting the indefinite article --a-- immediately before "whole". Accordingly, the objection to claim 72 should be withdrawn.

The Rejections of the Claims Under 35 U.S.C. § 112, First Paragraph, Should Be Withdrawn

Claims 62-94 have been rejected under 35 U.S.C. § 112, first paragraph. Claims 62, 63, 67, 68, 72-74, 87, and 88 have been amended. Claims 80-86 have been canceled. This rejection is respectfully traversed.

Written Description

Claims 62-94 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written description. The Office Action indicates that the claims are directed to methods and a plant comprising transforming a plant with a first nucleotide sequence and a second nucleotide sequence. The Office Action further indicates that the first nucleotide sequence encodes any NADPH-thioredoxin reductase, hybridizes under stringent conditions to the sequence set forth in SEQ ID NO: 24 or has at least 95% identity to the sequence set forth in SEQ ID NO: 24. The Office Action further indicates that the second nucleotide sequence encodes any thioredoxin h, hybridizes under stringent conditions to the sequence set forth in SEQ ID NO: 13, or has at least 95% identity to o the sequence set forth in SEQ ID NO: 13.

Applicants respectfully disagree with this characterization of the claims. In contrast to the view of the Office Action, claims 62-79 and 87-94 are directed to a first nucleotide sequence encoding an NADPH-thioredoxin reductase and a second nucleotide sequence encoding a thioredoxin h, the first nucleotide sequence is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 24;

b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 25;

c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequence set forth in SEQ ID NO: 24;

d) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO: 24; and

e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d) . . . .

Furthermore, of claims 62-79 and 87-94, only claims 63, 68, 74, and 88 further limit the second nucleotide sequence to a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 13;

b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 14;

c) a nucleotide sequence having at least 95% sequence identity to the coding sequence of the nucleotide sequences set forth in SEQ ID NO: 13;

d) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO: 13; and

e) a nucleotide sequence that is complementary to the nucleotide sequence of a), b), c), or d).

Finally, claim 80 and dependent claims 81-86 are directed to a first nucleotide sequence encoding an NADPH-thioredoxin reductase and a second nucleotide sequence encoding a thioredoxin *h*. These claims, however, do not recite further limitations concerning either the first nucleotide sequence or the second nucleotide sequence.

In addition, the Office Action indicates on page 3 of that "Applicants only disclose the nucleotide sequences set forth in SEQ ID NO: 24 or 13." This statement is incorrect. In fact, Applicants disclose in the instant application 10 nucleotide sequences that encode thioredoxin *h* proteins and 3 nucleotide sequences that encode NADPH-thioredoxin reductases. Furthermore, of the 10 sequences encoding thioredoxin *h* proteins, at least 8 encode full-length thioredoxin *h* proteins. *See*, original claims 3-51, SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 18, 20, 22, and 24 of the Sequence Listing, and the specification, for example, at lines 1-5 of page 6, from line 31 of page 14 to line 5 of page 15, and at lines 19-24 of page 15, page 15.

Applicants respectfully remind the Examiner that he previously recognized that the instant application discloses more than a single thioredoxin *h* encoding nucleotide sequence and a single NADPH-thioredoxin reductase encoding nucleotide sequence. In the Office Action mailed February 23, 2004, the Examiner required Applicants to elect one of four groups (Groups

I-IV) of claims for examination and further required a sequence election within the elected group. *See*, the Office Action mailed February 23, 2004.

The Office Action indicates that Applicants do not identify essential regions of the NADPH-thioredoxin reductase or thioredoxin *h* proteins encoded by SEQ ID NO: 24 and SEQ ID NO: 13, respectively, any sequences that hybridize to SEQ ID NO: 24 or SEQ ID NO: 13, nor any sequences that have at least 95% sequence identity to SEQ ID NO: 24 or 13 and encode a protein with the same activity as an NADPH-thioredoxin reductase encoded by SEQ ID NO: 24 or a protein with the same activity as an thioredoxin *h* encoded by SEQ ID NO: 13.

The Office Action concludes that the specification fails to provide an adequate written description to support the breadth of the claims. The Office Action bases this conclusion on two assertions. First, the Office Action asserts that Applicants fail to describe a representative number of polynucleotide sequences encoding an NADPH-thioredoxin reductase or thioredoxin *h* falling within the claimed genus of polynucleotides which hybridize to SEQ ID NO: 24 or SEQ ID NO: 13 or which have at least 95% sequence identity to SEQ ID NO: 24 or SEQ ID NO: 13. Second, the Office Action asserts that Applicants fail to describe the structural features of the claimed genus of polynucleotides. The Office Action cites *Regents of the University of California v. Eli Lilly and Co.*, 43 U.S.P.Q. 2d 1398 (Fed. Cir. 1997) in support of this conclusion.

In contrast to the position of the Office Action, the specification provides a sufficient written description to convey to one skilled in the relevant art that the inventors, at the time when the application was filed, had possession of the claimed invention, including claims directed to variants of the exemplified NADPH-thioredoxin reductase and thioredoxin *h* sequences. The Examiner is respectfully reminded that one skilled in the art would be familiar with the journal articles, patents, published patent applications, NADPH-thioredoxin reductase and thioredoxin *h* sequence accessions in public sequence databases, and other references in the relevant art, including, but not limited to, the references cited in the specification and in Applicants' Information Disclosure Statements.

At the time when the present application was filed, structural features of plant NADPH-thioredoxin reductases were known to those skilled in the art. In fact, the crystal structure of a plant NADPH-thioredoxin reductase had been reported. Dai *et al.* ((1996) *J. Mol. Biol.* 264:1044-1057; see Applicants' Information Disclosure Statement submitted concurrently herewith) teach the crystal structure of a plant NADPH-thioredoxin reductase from *Arabidopsis thaliana* at 2.5 Å resolution and compare this crystal structure with the known crystal structure of an *E. coli* NADPH-thioredoxin reductase. Furthermore, Dai *et al.* provide a multiple amino acid sequence alignment (p. 1051) of the NADPH-thioredoxin reductases from *Arabidopsis thaliana*, with bacterial and fungal NADPH-thioredoxin reductases, including NADPH-thioredoxin reductases from *Escherichia coli*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Streptomyces coelicolor*, *Streptomyces clavuligerus*, *Haemophilus influenzae*, *Coxiella burnetii*, *Eubacterium acidaminophilum*, *Clostridium pasteurianum*, *Neurospora crassa*, *Penicillium chrysogenum*, and *Saccharomyces cerevisiae*. Dai *et al.* also teach conserved residues of NADPH-thioredoxin reductases, including residues that are absolutely conserved across the NADPH-thioredoxin reductases from 14 species and that these conserved residues occur within the FAD binding site, the NADPH binding site, in active site disulfide and its surroundings, and in internal hydrophobic clusters (see, Table 2, p. 1052). Similarly, Jacqout *et al.* ((1994) *J. Mol. Biol.* 235:1357-1363; see, Applicants' Information Disclosure Statement submitted March 22, 2002) disclose that the amino acid sequence identity between the *Escherichia coli* NADPH-thioredoxin reductase and each of two *Arabidopsis thaliana* NADPH-thioredoxin reductases is about 45%. Jacqout *et al.* further disclose that several motifs are conserved between the NADPH-thioredoxin reductases from *Arabidopsis thaliana* and *E. coli* including the FAD, central, and NADPH-binding domains, suggesting a similar folding of the proteins (p. 1357) Jacqout *et al.* additionally provide a multiple amino acid sequence alignment (p. 1359) of the NADPH-thioredoxin reductases from *Arabidopsis thaliana*, *E. coli*, *Steptomyces clavuligerus*, and *Clostridium pasteurianum* and teach conserved amino acid residues.

At the time when the present application was filed, structural features of plant thioredoxin *h* proteins were also known to those skilled in the art. For example, Gautier *et al.* ((1997) *Eur. J. Biochem.* 252:314-324; see, Applicants' Information Disclosure Statement submitted March 22, 2002) teach the nucleotide and amino acid sequences of two wheat thioredoxin *h* proteins, one from *Triticum aestivum* and the other from *Triticum durum* (p. 317), and an alignment of these two amino acid sequences with the amino acid sequences of 12 other NADPH-thioredoxin reductases from five plant species, including *Oryza sativa*, *Nicotiana tabacum*, *Arabidopsis thaliana*, *Brassica napus*, and *Glycine max* (p. 318). Gautier *et al.* further teach important structural and functional amino acids that are conserved in thioredoxin *h* sequences and that the cereal thioredoxin *h* proteins are highly conserved. Ishiwatari *et al.* ((1995) *Planta* 195:456-463; Applicants' Information Disclosure Statement submitted March 22, 2002) teach the nucleotide and amino acid sequences of a rice phloem sap thioredoxin *h* (p. 458), provide a multiple amino acid sequence alignment of the rice phloem sap thioredoxin *h* with the amino acid sequences of plant, bacterial, fungal, and animal thioredoxin *h* proteins, and identify conserved amino acid residues (p. 459). Brugidou *et al.* ((1993) *Mol. Gen. Genet.* 238:285-293; see, Applicants' Information Disclosure Statement submitted March 22, 2002) teach the nucleotide and amino acid sequences of a tobacco thioredoxin *h* (p. 288) and provide a multiple amino acid sequence alignment of the tobacco thioredoxin *h* with the amino acid sequences of plant, bacterial, fungal, and animal thioredoxin *h* proteins (p. 289). Bréhélin *et al.* ((2000) *J. Biol. Chem.* 275:31641-31647; see, Applicants' Information Disclosure Statement submitted March 22, 2002) provide a multiple amino acid sequence alignment of five *Arabidopsis thaliana*, two *Saccharomyces cerevisiae* and one *Chlamydomonas reinhardtii* thioredoxin *h* proteins and teach conserved amino acid residues and secondary structures, including α-helices and β-sheets (p. 31642).

While claims 62-79 and 87-94 recite that the first nucleotide sequence encodes an NADPH-thioredoxin reductase, Applicants, have amended claims 62, 67, 73, and 87 to point out more distinctly that the nucleotide sequences of parts (c) and (d) encode proteins comprising NADPH-thioredoxin reductase activity. While claims 63, 68, 74, and 88 recite that the second nucleotide sequence encodes a thioredoxin *h*, Applicants have amended claims 63, 68, 74, and 88

to point out more distinctly that the nucleotide sequences of parts (c) and (d) encode proteins comprising thioredoxin *h* activity. Applicants have additionally amended part (d) of claims 62, 63, 67, 68, 73, 74, 87, and 88 to recite specific stringent conditions and to recite that "a nucleotide sequence that hybridizes under stringent conditions to *the complement of* the nucleotide sequence set forth in SEQ ID NO: . . ." (emphasis added)

The amendments to claims 62, 63, 67, 68, 73, 74, 87, and 88 were made for the purposes of furthering prosecution and not to limit the scope of Applicants' claimed invention. Furthermore, the amendment of dependent claims 63, 68, 74, and 88 does not, and is not intended to limit the scope of claims 62, 64-67, 69-73, 75-79, 87, and 89-94.

Amended claims 62, 67, 73, and 87, and their respective dependent claims recite, as *Lilly* requires, the functional and structural features of the first nucleotide sequence. Amended claims 62, 67, 73, and 87 recite that with respect to the first nucleotide sequence, the fragment and variant nucleotide sequences encode NADPH-thioredoxin reductase proteins comprising NADPH-thioredoxin reductase activity or are the complements of such nucleotide sequences. Similarly, amended claims 63, 68, 74, and 88 additionally recite, as *Lilly* requires, the functional and structural features of the second nucleotide sequence. Amended claims 63, 68, 74, and 88 additionally recite that with respect to the second nucleotide sequence, the fragment and variant nucleotide sequences encode thioredoxin proteins comprising thioredoxin *h* activity or are the complements of such nucleotide sequences.

In contrast to the position of the Office Action, the specification provides adequate description of the subject matter of the amended claims and their respective dependent claims, so as to reasonably convey to one skilled in the relevant art that Applicants had possession of the invention as claimed. In particular, the specification further discloses on pages 14-17 that the invention encompasses fragments and variants of the disclosed nucleotide sequence, wherein such fragments and variants encode proteins comprising NADPH-thioredoxin reductase activity or thioredoxin *h* activity. Finally, one skilled in the art would be familiar with the teachings of the references that are discussed above concerning important structural and functional characteristics of NADPH-thioredoxin reductase and thioredoxin *h* proteins. Accordingly, the

subject matter of amended claims 62, 63, 67, 68, 73, 74, 87, and 88 and their respective dependent claims is adequately described in the instant specification so as to reasonably convey to one of ordinary skill in the relevant art that, at the time of the invention, Applicants had possession of the claimed invention. The written description requirement of 35 U.S.C. §112, first paragraph, has been satisfied.

In summary, in view of the amendments and above remarks, claims 62-79 and 87-94 satisfy the written description requirement of 35 U.S.C. § 112, first paragraph, and the Examiner is respectfully requested to withdraw the rejection.

Enablement

Claims 62-94 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement.

The Office Action indicates that the specification is enabled for a method of decreasing the number of disulfide bonds of storage proteins in a plant or part thereof comprising transforming a plant with a nucleotide sequence comprising SEQ ID NO: 24 encoding a NADPH-thioredoxin reductase and a nucleotide sequence comprising SEQ ID NO: 13 encoding a thioredoxin *h* wherein the expression of both nucleotide sequences in grains reduces the disulfide status of storage protein, and a plant transformed with said nucleotide sequences.

The Office Action indicates that the claims are drawn to a method of decreasing the number of disulfide bonds of storage proteins in a plant or part thereof wherein the hardness of a grain is increased, a method for improving the digestibility of grain, a method for improving grain for processing or a transformed plant comprising transforming a plant with a nucleic acid sequence encoding any NADPH-thioredoxin reductase, a nucleic acid sequence comprising SEQ ID NO: 24, or wherein the nucleotide sequence hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO: 24 or is 95% identical to the sequence set forth in SEQ ID NO: 24 and comprising transforming said plant a nucleic acid sequence encoding any thioredoxin *h*, a nucleic acid sequence comprising SEQ ID NO: 13, or wherein the nucleotide sequence hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID

NO: 13 or is 95% identical to the sequence set forth in SEQ ID NO: 13, wherein the nucleotide sequences are operably linked to a promoter, wherein the process is wet milling, grinding, steam flaking of dry grind ethanol production.

Applicants again disagree with the characterization of the claims in the Office Action as discussed above in Applicants' remarks above concerning the rejections of claims for lack of adequate written description. For the sake of brevity, Applicants will not repeat those remarks here and instead refer the Examiner to their remarks above.

Further to those remarks, Applicants discuss below additional mischaracterizations of the claims that were made on pages 6 and 7 of the Office Action. On page 6, the Office Action indicates that "[t]he claims are drawn to a method for altering disulfide status of storage proteins in a plant or part thereof wherein hardness of a grain is increased . . . ." While claims 62-66 and 80-86 are directed to a method for altering disulfide status of storage proteins in a plant or part thereof, these claims are not solely limited to "wherein hardness of a grain is increased." Applicants do note that dependent claim 85, includes the recitation "wherein the hardness of said grain is increased or decreased." However, claims 62-66, 80-84, and 86 do not recite any limitation concerning grain hardness, whether it be increased or decreased. In the paragraph that bridges pages 6-7, the Office Action mischaracterizes the claims as including the limitation "wherein the processing is wet milling, grinding, steam flaking or dry grind ethanol production." Although claim 76 includes the recitation "processing is wet milling", claim 77 includes the recitation "processing is grinding", claim 78 includes the recitation "processing is steam flaking", and claim 79 includes the recitation "processing is dry grind ethanol production", claims 62-74 and 80-84 do not include any such processing limitation.

The Office Action asserts that the specification does not provide reasonable enablement for claims drawn to a method for altering the disulfide status of storage proteins in a plant or part thereof, wherein grain hardness is increased, a method for improving the digestibility of grain, a method for improving grain for processing or a transformed plant comprising transforming a plant with a first and a second nucleotide sequence encoding any NADPH-thioredoxin reductase and thioredoxin *h*, respectively, or wherein the first nucleotide sequence comprises SEQ ID

NO: 24 or has at least 95% sequence identity to SEQ ID NO: 24 or hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO: 24 or the second nucleotide sequence comprises SEQ ID NO: 13 or has at least 95% sequence identity to SEQ ID NO: 13 or hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO: 13. The Office Action concludes the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. In support of this position, the Office Action cites *In re Wands* 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988).

As discussed above, parts (c) and (d) of claims 62, 67, 73, and 87 have been amended to recite that the first nucleotide sequence encodes a polypeptide comprising NADPH-thioredoxin reductase activity. Part (d) of these claims has been further amended to recite that the stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC. Similarly, parts (c) and (d) of claims 63, 68, 74, and 88 have been amended to recite that the first nucleotide sequence encodes a polypeptide comprising thioredoxin *h* activity. Part (d) of these claims has been further amended to recite that the stringent conditions comprise hybridization at 37°C in a solution comprising in 50% formamide, 1 M NaCl, and 1% SDS, and at least one wash at 60°C in a solution comprising 0.1X SSC.

In contrast to the conclusions of the Office Action, the specification provides sufficient guidance to make and identify the nucleotide molecules encompassed by the claims. In particular, Applicants have provided the nucleotide sequences of SEQ ID NO: 24 and SEQ ID NO: 13 and the amino acid sequences of SEQ ID NO: 25 and SEQ ID NO: 14. The claimed nucleotide sequences vary from this sequence by structural parameters (*i.e.*, at least 95% identity to SEQ ID NO: 24 or 13, or hybridizes to the complement of SEQ ID NO: 24 or 13 under defined stringent conditions) that can be determined by those of ordinary skill in the art. While methods for sequence alignments, sequence comparisons, determining percent sequence identity, and hybridization are within the knowledge of one of ordinary skill in the art, additional guidance for is set forth in the specification on pages 19-27.

Moreover, the nucleotide sequences of the invention encode polypeptides comprising NADPH-thioredoxin reductase activity or thioredoxin *h* activity. Such nucleotide sequences include those that are fragments and variants of SEQ ID NO: 24 and that encode polypeptides comprising NADPH-thioredoxin reductase activity and those that are fragments and variants of SEQ ID NO: 13 and that encode polypeptides comprising thioredoxin *h* activity. Methods for assaying whether the nucleotide sequences encode proteins comprising NADPH-thioredoxin reductase activity or thioredoxin *h* activity are known in the art and are also provided in the instant specification on page 17 at lines 18-21. Accordingly, based on the guidance in the specification, one of ordinary skill in the art would be able to make and use the nucleotide molecules of Applicants' claimed invention.

The Federal Circuit has repeatedly stated that enablement is not precluded by the necessity for some experimentation, so long as the experimentation needed to practice the invention is not undue. *In re Wands* 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988). Furthermore, a considerable amount of experimentation is permissible, if it is merely routine, or if the specification provides a reasonable amount of guidance in which the experimentation should proceed. *Id.*

Applicants stress that when evaluating the quantity of experimentation required, the court looks to the amount of experimentation required to practice a single embodiment of the invention, rather than the amount required to practice every embodiment of the invention. For example, in *Wands*, the claims at issue were drawn to immunoassay methods using any monoclonal antibody having a binding affinity for HbsAg of at least 10^{-9} M. The USPTO had taken the position that the claim was not enabled as it would take undue experimentation to make the monoclonal antibodies required for the assay. The Federal Circuit reversed, and held that the claims were enabled, as the amount of experimentation required to isolate monoclonal antibodies and screen for those having the correct affinity was not undue. *Id.* Clearly, the Federal Circuit did not contemplate that every antibody useful in the methods of the claim must be identified. Rather, the court considered the amount of experimentation required to identify one or a few monoclonal antibodies having the required affinity.

In the instant case, the quantity of experimentation required to practice the invention amounts to two steps, identifying a nucleotide sequence that comprises at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 24, or hybridizes to SEQ ID NO: 24 under defined stringent conditions, and then assaying the protein encoded thereby for functional activity. For the embodiments of the invention encompassed by claims 63, 68, 74, and 88, the quantity of experimentation required to practice the invention amounts to two additional steps, identifying a nucleotide sequence that comprises at least 95% sequence identity to the nucleotide sequence of SEQ ID NO: 13, or hybridizes to SEQ ID NO: 13 under defined stringent conditions, and then assaying the protein encoded thereby for functional activity. Thus, ample guidance is provided to allow one of skill in the art to identify additional nucleotide sequences encompassed by the claims. Consequently, contrary to the conclusions of the Office Action, the quantity of experimentation necessary and the amount of guidance presented in the specification is sufficient to enable Applicants' claimed invention. Accordingly, Applicants submit that claims 62-79 and 87-94 are enabled under 35 U.S.C. §112, first paragraph.

The Office Action indicates that Applicants used the method of Boisen and Fernandez ((1997) *Animal Feed Sci. Technol.* 68:277-286) to measure the digestibility of the maize kernels produced by the methods of the invention. The Office Action asserts that Applicants have not improved the digestibility or grain processing of corn kernels or stated the relationship between the EDDM% assay and digestibility and processing of grains. The Office Action further asserts that it is not clear how Applicants can claim methods of digestibility and processing of grains when Applicants have not measured animal excreta from animals that have been fed Applicants' maize plants or kernels or analyzed the processing characteristics of said maize kernels. The Office Action, however, fails to cite any references to support these assertions.

Notwithstanding these assertions of the Office Action, those of skill in the art recognize that usefulness of in vitro assays for determining digestibility. The specification discloses on page 11 at lines 10-20 and 27-31:

> Digestibility can be estimated using in vitro assays, which is routinely done to screen large numbers of different food ingredients and plant varieties. In vitro techniques, including assays with rumen inocula and/or enzymes for ruminant livestock (e.g., Tilley, J.M.A., and Terry, R.A. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111; Pell, A.N., and Schofield, P. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. J. Dairy Sci. 76:1063-1073) and various combinations of enzymes for monogastric animals reviewed in Boisen and Eggum (1991) are also useful techniques for screening transgenic materials for which only limited sample is available. (See Boisen, S., and Eggum, B.O. 1991. Critical Evaluation of in vitro methods for estimating digestibility in simple-stomach animals. Nutr. Res. Rev. 4:141-162).
>
> . . . .
>
> Methods for assessing the digestibility and/or energy availability of animal feeds are known in the art. Such methods can be used to determine the digestibility and/or energy availability of the plant parts of the invention, particularly grain. See, for example, Boisen and Fernandez (1997) *Animal Feed Sci. Technol. 68*:277; herein incorporated by reference.

While the Office Action may express doubt concerning the applicability of the in vitro method of Boisen and Fernandez without citing any references in support of this position, Boisen and Fernandez disclose the general validity of their method in a peer-reviewed journal article as evidenced by a significant correlation ($r^2 = 0.87$) between in vitro digestibility of energy values determined by their method and in vivo digestibility of energy values in pigs for 33 feedstuffs (p. 283). Further evidence of the general validity of the in vitro method disclosed by Boisen and Fernandez is the adoption by Denmark of a national method for assessing the energy value of compounded feeds that is based on the general prediction equation that was developed from this comparison of in vitro and in vivo results (p. 283).

Applicants' invention is directed to compositions and methods for altering the disulfide status of proteins. In the instant specification, Applicants have disclosed that the overexpression in mature maize kernels of enzymes that are involved in disulfide reduction—particularly NADPH-thioredoxin reductase and/or thioredoxin *h*—increases the digestibility of such maize kernels (see Example 11, pp. 51-55). Those of ordinary skill in the art would recognize that

alterations in disulfide status that increase the digestibility of mature grains will also improve such grains for processing in situations known to benefit from disulfide reduction. While those of ordinary skill in the art understand that wet milling involves the use of sulfhydryl reductants to reduce protein cross-linking, the specification discloses on page 7 at lines 12-17:

> The corn wet-milling process requires steeping with large amounts of sulfur-reducing chemicals (e.g., sulfur dioxide) to reduce the disulfides present in corn kernels and thereby maximize starch yield (Hoseney, R.C. 1994. Principles of Cereal Science and Technology, second edition. Am. Assoc. Cereal Chemists, St. Paul, Minnesota). By decreasing the disulfide status of grain, the amount of sulfur-reducing chemicals used in wet milling can be decreased.

Those of ordinary skill in the art recognize that an important determinant of the rigidity or hardness of grains is the degree of disulfide cross-linking of proteins and that decreasing protein cross-linking can reduce the amount of energy required for grinding, steam-flaking and dry-grind ethanol production, as well as increase the efficiency of these processes. The instant specification discloses on page 8 at lines 6-17:

> The response to steam-flaking of corn and sorghum grain is negatively correlated with protein disulfide content (Blackwood, R.B., and Richardson, C.R. 1994. Steam-flaking and grain source effects on disulfide bonds in grain sorghum and corn. In: Animal Science and Food Technology Research Report 1994. Agricultural Sciences and Natural Resources Technical Report No. T-5-342. Texas Tech University, Lubbock, Texas, pp. 49-51). For corn or sorghum with lower degree of protein disulfide cross-linking, the extent of disulfide rearrangements during processing is reduced, which allows for higher and more uniform response to steam-flaking, and which can be expected to reduce the energy required in steam-flaking, as well as in grinding. Furthermore, the feed quality of the grain will be improved by reduced endosperm rigidity, allowing for reduced particle size of ground corn under fixed grinding regime.

Furthermore, those of ordinary skill in the art will recognize that grain with increased digestibility will be also improved for dry grind ethanol production due to improved energy availability for fermentation. In fact, the specification discloses on page 8 at lines 18-22:

> In addition, dry grind ethanol production is improved by the use of the invention described herein. This improvement may be due to increased fermentable starch available for ethanol production. Dry grind ethanol production may also be improved as a result of the increased digestibility, and therefore increased value, of fermentation by-products such spent brewer's grain or thin stillage.

Thus, while the Office Action may assert that it is not clear how Applicants can claim methods of improving digestibility and processing of grains when Applicants have not measured animal excreta, those of ordinary skill in the art will recognize that the instant specification is fully enabling for Applicants' claimed methods for altering the disulfide status of storage proteins, improving the digestibility of grain, and improving grain for processing, and for Applicants' claimed transformed plants.

The Office Action concludes that it cannot be predicted by one of ordinary skill in the art that sequences that are at least 95% identical, or hybridize under stringent conditions, to SEQ ID NO: 24 or 13 will encode a protein with the same activity as either a protein encoded by SEQ ID NO: 24 or 13 and cites the teachings of the Bowie *et al.* ((1990) *Science* 247:1306-1310) and McConnell *et al.* ((2001) *Nature* 411:709-713) references in support of this view.

The Office Action, however, fails to indicate that the Bowie *et al.* reference teaches that guidance on allowed amino acid substitutions can be obtained through alignments with other members of a protein family. Furthermore, the Office Action does not indicate that the McConnell *et al.* reference disputes this teaching of Bowie *et al.* While those of the ordinary skill in the art do not need guidance to conduct such sequence alignments of NADPH-thioredoxin reductase and thioredoxin *h* protein family members, the specification provides additional guidance on pages 21-27. Furthermore, as discussed above, structural features of plant NADPH-thioredoxin reductase and thioredoxin *h* proteins were known to those skilled in the art at the time the application was filed. For the sake of brevity, Applicants will not repeat here their discussion concerning the known structural characteristics, sequence alignments, and conserved amino acids of NADPH-thioredoxin reductase and thioredoxin *h* proteins, and instead

refer the Examiner to their discussion above of the teachings of the Dai *et al.*, Jacqout *et al.*, Gautier *et al.*, Ishiwatari *et al.*, Brugidou *et al.*, Bréhélin *et al.* references.

The Office Action asserts that Applicants have not disclosed how one makes or isolates any of the sequences that are encompassed by Applicants' broad claims and further have not taught which regions of the respective polynucleotides can be used to amplify any of said polynucleotides or which regions can be used as a probe to isolate any of said polynucleotide sequences. Those of ordinary skill in the art know how to design and produce primers for PCR amplification and probes for hybridization, particularly when the sequences of a polynucleotide and the polypeptide it encodes are provided. In addition to the nucleotide and amino acid sequences set forth in SEQ ID NOs: 1-25, the instant specification provides additional guidance for PCR amplification, primers, hybridization, and probes on pages 14, 15, and 18-21. Furthermore, the Examiner is again reminded that those of ordinary skill in the would be familiar with the teachings on the structural characteristics, sequence alignments, and conserved amino acids of NADPH-thioredoxin reductase and thioredoxin *h* proteins of the Dai *et al.*, Jacqout *et al.*, Gautier *et al.*, Ishiwatari *et al.*, Brugidou *et al.*, Bréhélin *et al.* references, the other references cited in the specification and Applicants' Information Disclosures Statements, and those NADPH-thioredoxin reductase and thioredoxin *h* sequences that were disclosed in public sequence databases prior to the filing of the instant application.

The Office Action indicates that Applicants' claimed methods are not enabled for increasing the number of disulfide bonds in utilizing antisense and cosuppression technology. The Office Action asserts that sense and antisense constructs behave unpredictably when transformed into a plant. The Office Action cites the Colliver *et al.* reference ((1997) *Plant Mol. Biol.* 35:509-522) as teaching expression of an antisense bean chalchone synthase construct in bird's foot trefoil unexpectedly resulted in transformants with increased levels of chalcone synthase transcripts.

Unlike the Colliver *et al.* reference, Applicants' claimed invention involves expressing in plants polynucleotides encoding NADPH-thioredoxin reductase and thioredoxin *h* and not polynucleotides encoding bean chalcone synthase, an enzyme involved in secondary metabolic

pathways. NADPH-thioredoxin reductase and thioredoxin *h* are not known to be enzymes that are involved in secondary metabolic pathways in plants. Furthermore, Colliver *et al.* admit that their results are "*not consistent with general antisense effects* or with reported antisense modification of secondary metabolic pathways in which the level of detectable endogenous transcript has been noted to be significantly reduced." (p. 519, citation omitted) (emphasis added)

In the instant specification, Applicants provide not only the exemplified nucleotide sequences set forth in claims 24 and 13, but also sufficient guidance for one of ordinary skill in the art to make and use the invention as claimed in any plant, including methods that involve antisense suppression and/or cosuppression. While antisense suppression and cosuppression methods are known to those of ordinary skill in the art, the specification provides additional guidance on pages 39 and 40, including the teachings of U.S. Patent Nos. 5,283,184 and 5,034,323, which have been incorporated by reference into the specification. Thus, the claims are enabled for increasing the number of disulfide bonds by utilizing antisense and cosuppression technology.

In view of the amendments and above remarks, it is apparent that those of skill in the art would be able to practice the present claims without undue experimentation. Accordingly, the enablement rejection of claims 62-79 and 87-94 should be withdrawn.

The Rejection of the Claims Under 35 U.S.C. § 102(e) Should Be Withdrawn

Claims 62-94 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Lanahan (December, 1998, WO 00/36126). Claims 62, 63, 67, 68, 72-74, 87, and 88 have been amended. Claims 80-86 have been canceled. This rejection is respectfully traversed.

While the Office Action discusses the various teachings of WO 00/36126 and concludes that WO 00/36126 anticipates Applicants' claimed invention, Applicants take no position at this time regarding the specific teachings of WO 00/36126. Instead, Applicants respectfully submit that this rejection of the claims under 35 U.S.C. § 102(e) is improper because the international

filing date of WO 00/36126 is December 15, 1999. The Examiner is respectfully reminded that as is indicated in M.P.E.P. § 2136.01, "references based on international applications that were filed prior to November 29, 2000 are subject to the former (pre-AIPA) version of 35 U.S.C. 102(e)." The pre-AIPA version of 35 U.S.C. 102(e) reads as follows:

> A person shall be entitled to a patent unless
>
> . . . .
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

Unlike the latest version of 35 U.S.C. § 102(e), the pre-AIPA version of 35 U.S.C. § 102(e) does not authorize the Office to use a published international application as the basis for a rejection of a claim. Thus, it is improper to use WO 00/36126 as the basis for a rejection of the claims under 35 U.S.C. § 102(e).

In view of the remarks, it is submitted that the rejection of the claims under 35 U.S.C. § 102(e) should be withdrawn.

CONCLUSION

In view of the above amendments and remarks, Applicants submit that the rejections of the claims under 35 U.S.C. §§ 102(e), and 112, first paragraph, are overcome. Applicants respectfully submit that this application is now in condition for allowance. Early notice to this effect is solicited. In any event, the Examiner is respectfully requested to enter the above amendments for the purpose of furthering prosecution.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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Karyn Gramm